Plasma Lipid Profiles, Milk Yield and Milk Fat Concentration of Lactating Holstein Fed Rumen-Protected Choline

by Dian W. Harjanti

Plasma Lipid Profiles, Milk Yield and Milk Fat Concentration of Lactating Holstein Fed Rumen-Protected Choline

Indra S. Hesti, D. Trizana, A. Wicaksono, P. Sambodho, Christina B. Soejono, Sri A. B. Santoso, A. Subrata, and Dian W. Harjanti*

***Abstract* — Choline plays an essential role in fat metabolism in the liver. The purpose of this study was to evaluate the effects of addition rumen-protected choline (RPC) on plasma lipid profiles (concentrations of plasma cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides), milk yield and milk fat concentration in lactating Holstein. Eight of lactating Holstein (in the 2nd lactation period and 456 ± 31 kg of body weight) used. The cows were fed two diets in a crossover experiment for 90 days. The first diet was total mixed ration (TMR) containing Napier grass and concentrate (40:60) as control diet, and the second diet was TMR added with RPC as choline chloride 30 g/head/d (choline chloride 60% corn-cob as equal to 0.02% BW$^{0.75}$). The data was analyzed with one simple T-test using SPSS ver.21. The results showed that addition RPC in the diet did not significantly influence (p>0,05) the concentrations of plasma lipid profiles. The inclusion of RPC in the diet increased (p<0.05) milk yields up to 0.38 kg/d and increased milk fat concentration up to 0.35%/d. Thus, choline chloride could be added in the diets of lactation cows to increase milk yield and milk fat.**

***Keywords* — choline chloride, plasma lipid, milk yield, milk fat, dairy cows.**

I. Introduction

In the tropic region, milk yield of dairy cow is relatively low and could not meet the total consumption need. The quality of feeding is important to improve the quantity and quality of milk produced. Choline has been classified as one of the B-complex vitamins, but it's determine was not complied into the definition of vitamins whose had enzyme co-factors [24]. Choline is formed as lecithin on feed and part of phosphatidylcholine whose the main phospholipid in cell membrane, lipoproteins, and milk fat globule on dairy cows [15]. Choline plays an essential role in fat metabolism in the liver by increasing the utilization of fatty acids in the liver. Moreover, choline contributes in lipid metabolism that serve Acetyl CoA as the precursor of cholesterol synthesis [10], as phosphatidylcholine (the main part of phospholipid that used in lipid transport) and as methyl group donor in metabolism (methionine, folic acids and vitamin B12) [24]. Methionine is used in very low density lipoprotein (VLDL) synthesis in liver then will be converted into LDL in blood. Methylation pathways form phosphatidylcholine, the main phospholipid of HDL. Vitamin B12 is needed as co-enzyme in methylation pathways and used to be precursor of gluconeogenesis from propionate. Highly energy efficiency implicated to increasing milk yield and highly lipid anabolism implicated to increasing milk fat. Because of that, choline become to be feed additive that need in dairy cows feed. Choline had been fully degraded in the rumen because of that choline have to be protected as Rumen Protected Choline (RPC) or choline chloride 60% corn-cob (contained 60% choline chloride mixed with 40% corn-cob as carrier) that choline can pass through reticulorumen and absorbed into small intestine [4,15]. Addition of choline chloride in feed is expected to optimize methyl group metabolism in order to prevent negative energy balance which can improve the increasing milk yield and to optimize lipid metabolism that the transport of fat milk precursor to the mammary gland will increase which the indicator is lipid plasma (cholesterol, triglycerides, LDL, and HDL in blood.

We hypothesized that addition choline chloride can improve the energy metabolism in order to improve the milk yield of dairy cows and increase plasma lipid within normal range in order to improve the milk fat of dairy cows. This research is expected that choline chloride can be incorporated into feed and could improve total milk production and milk fat.

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II. Materials and Methods

A. Animals diets

This research was used eight of Lactating Holstein cows (61 to 91 days in milk; 3th lactation period; 13.66 ± 1.26 kg/head/d of milk; and 456 ± 31 kg of BW = 99 ± 5 kg BW$^{0.75}$) and held in dairy farm on Central Java, Indonesia. The proximate analysis of diet was listed in Table 1. The diet was total mixed ration (TMR) with the ingredients were Napier grass and concentrate (40:60) which was assumed from the National Research Council [12], as shown in Table 2. Additive feed was RPC as choline chloride 60% corn-cob 30 g/head/d (as equal to 0.02% BW$^{0.75}$) referred by research before about RPC [9,15,23]. The

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TABLE I: THE PROXIMATE ANALYSIS OF DIETS INGREDIENTS

Nutrient composition	Napier grass	Concentrate
DM[a] (%)	15.32	88.39
TDN (%)	57.33[b]	69.54[c]
CP[d] (%)	8.01	16.70
CF[a] (%)	33.06	19.92
EE[a] (%)	2.72	4.55

[a]Nutrient and Feed Science Laboratory, Faculty of Animal and Agricultural Sciences Diponegoro University, (2015).
[b]TDN count based on Hartadi et al., (1980).
[c]TDN count based on Sutardi, (1979).
[d]Ecology and Plant Production Laboratory, Faculty of Animal and Agricultural Sciences, Diponegoro University, (2015).
DM : dry matter
TDN : total digestible nutrient
CP : crude protein
CF : crude fiber
EE : ether extract

RPC was purchased from Feed Grade Vitamins (Hebei Kangdali Pharmaceutical Co., Ltd, Hebei, China). The cows were fed two diets in a crossover experiment for 90 days (throughout into 2 periods) that consisted of 30 days of dietary adaptation and 60 days of dietary treatment. The diet treatments consisted of control diet (TMR) and RPC diet (TMR with choline chloride). Feeding of Napier grass was given twice per day on 08.00 a.m. and 04.00 p.m. then concentrate was given 4 times on 04.00 a.m., 07.00 a.m., 01.00 p.m. and 03.00 p.m. Adding RPC was given in the morning after 2nd concentrate on 07.00 a.m. had done. The water available was ad-libitum with nipple cup.

B. Sample collection and analysis

Blood samples were collected every 30 days and were taken from jugular vein using non EDTA tubes then were stored in cooling box. Serum was gotten from centrifuges on 2060 × g for 15 min at 5°C. Lipid plasma was analyzed using hemodialysis method with standardization value of analyzer (Caretium Biochemistry Analyzer). Standard value for cholesterol was 138 mg/dl that ranged 96-179 mg/dl, triglyceride was 104 mg/dl that ranged 73-135mg/dl, and HDL was 79 mg/dl that ranged 55-102 mg/dl. Milk yield was collected every days in the morning milking at 04.00 a.m. and evening milking at 03.00 p.m. Milk fat was analyzed by lactoscan machine every 7 days of periods.

C. Calculations

The LDL was calculated by Friedewald equation which was known with cholesterol total level, HDL level and triglicerides level. LDL = Cholesterol Total – (HDL + Triglycerides/5)

III. STATISTICAL ANALYSIS

The mean values and standard errors of the mean (SEM) were calculated for all of the data. All of the data were analyzed with One Simple T-Test procedures in SPSS ver.21. Statistical significance was declared at $P \leq 0.05$, and a tendency to significance was declared at $0.05 < P \leq 0.20$.

TABLE II: THE INGREDIENT AND NUTRIENT COMPOTITION OF DIETS

Composition	Dietary Treatments			
	TMR diet		RPC diet	
	Napier grass	Concentrate	Napier grass	Concentrate
Ratio	40	60	40	60
DM[a] (%)	6.12	53.03	6.12	53.03
TDN (%)	22.93	41.72	22.93	41.72
CP[d] (%)	3.20	10.02	3.20	10.02
CF[a] (%)	13.22	11.95	13.22	11.95
EE[a] (%)	1.08	2.73	1.08	2.73
RPC (g/head/d)	0		30	

IV. RESULT

A. Dry matter intake

The average of DMI was not affected significantly by RPC diet. DMI of TMR diet was 13.50 ± 2.18 kg/head/d and 2.91 ± 0.42% BW. The DMI of RPC diet was 14,19 ± 1,98 kg/head/d and 3.09 ± 0.35% BW (showed in Table III.). The DMI of two treatments were ranging between 2.25 – 4.32% BW. The sufficiency of DMI is used for performance and milk production. Based on National Research Council [12] that the DMI of dairy cows ranged 2.25 – 4.32% BW. The averages DMI showed that RPC diet tended to increase 0.69 ± 0.20 kg/head/d ($P = 0.2$) and 0.18 ± 0.07% BW ($P = 0.2$) than TMR diet. The role play of choline in increasing utilization efficiency of energy tended to cause the increasing energy metabolism. DMI was not affected by metabolism rate but was affected by type of feed and digestibility. Choline plays an important role in the regulation of carbohidrate conversion into CO2 and ATP [13]. The liver glycogen concentration increased linearly while increasing intake of choline chloride that to assist the formation of glycogen from hydrolysis to be used as a source of glucose in the liver become faster that affected to increasing milk yield [4,14]. Addition 40 g/head/d RPC increased 0.3 kg/d DMI (Davidson, 2008) the addition 25 g/head/d RPC (50% choline chloride) has been significantly increased DMI of 2.2 kg/head/d and milk yield of 4.4 kg/d [6].

B. Ether extract intake

The average of EEI was not affected by RPC diet. EEI of TMR diet averaged 512 ± 83 g/head/d and 0.11 ± 0.18% BW. EEI of RPC diet averaged 543 ± 81 g/head/d and 0,12 ± 0.17% BW (has been shown in Table III.). It showed that the EEI with RPC diet increased numerically 31.25 ± 0.2 g/head/d ($P = 0.19$) and 0.01± 0.01% BW ($P = 0.18$). EEI has not affected significantly due to DMI which not significantly affected. The EEI affected increasing lipid metabolism rapidly so transfer of fatty acid to mammary gland will increase. Choline which provides a methyl group donor in the synthesis of carnitine is an essential component for the oxidation of fatty acids so that the mobilization of non esterified fatty acids (NEFA) from feed in blood to the liver increases and does not accumulate in the liver. EEI can increase cholesterol and LDL levels in blood [18]. Feed intake is a factor that determine of function, respond and nutrient feed using [2].

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TABEL III: EFFECTS OF CHOLINE CHLORIDE TO DRY MATTER INTAKES AND ETHER EXTRACT INTAKES

Parameters	Treatments	
	TMR diet	RPC diet
DMI (kg/head/d)	13,50 ± 2,18	14,19 ± 1,98
DMI (% BW)	2,91 ± 0,42	3,09 ± 0,35
EEI (g/head/d)	512,38 ± 82,23	543,63 ± 79,81
EEI (% BW)	0,11 ± 0,18	0,12 ± 0,17

DMI = Dry Matter Intake; EEI = Ether Extract Intake.

TABEL IV: EFFECT OF CHOLINE CHLORIDE TO PLASMA LIPID

Parameters	Treatments	
	TMR diet	RPC diet
Cholesterol level (mg/dl)	159,37 ± 21,02	161,43 ± 21,52
Trygliceride (mg/dl)	70,14 ± 6,76	69,16 ± 3,53
LDL level (mg/dl)	74,77 ± 16,94	80,21 ± 24,67
HDL level (mg/dl)	70,52 ± 11,76	67,48 ± 15,62

LDL = Low density lipoprotein; HDL = High density lipoproteins.

TABEL V: EFFECT OF CHOLINE CHLORIDE TO MILK YIELD AND MILK FAT

Parameters	Treatments	
	TMR diet	RPC diet
Milk yield (kg/d)	$13{,}84 \pm 2{,}41^{a}$	$14{,}29 \pm 2{,}90^{b}$
Milk yield 4% FCM (kg/d)	$13{,}36 \pm 2{,}63^{a}$	$14{,}53 \pm 3{,}12^{b}$
Milk fat (%)	$3{,}76 \pm 0{,}27^{a}$	$4{,}11 \pm 0{,}28^{b}$

C. *Cholesterol*

Cholesterol level in blood has not been affected by treatments. Cholesterol level of TMR diet averaged 159.37 ± 21 mg/dl and cholesterol level of RPC diet averaged 161.43 ± 22 mg/dl (as showed in Table IV.). Cholesterol level was in the normal value that ranged between 139-177 mg/dl [21]. Cholesterol level is influenced by the consumption of DMI and EEI that provides a source of energy for the precursor of cholesterol, named Acetyl CoA which is produced from glucose and fatty acids in the mitochondria and amino acid catabolism [10]. Cholesterol level with RPC diet was higher 1.06 ± 0.1 mg/dl (P = 0.6) in normal range than TMR diet although it was not affected significantly. The role play of choline was to improve supply acetyl-CoA which the main precursor for cholesterol synthesis and as a phospholipid (phosphatidylcholine) in lipoproteins that used in cholesterol transport through the blood flow. Cholesterol acts as the main compounds such as bile steroid body whose function as an emulsifier of fat so that the body can absorb it. Based on cholesterol was the main of all steroid compounds which had been synthesized in the body, one function of bile salts to emulsify fats into fatty acids that can be absorbed by the body [11]. Choline that formed phosphatidylcholine contained in bile function in maintaining the hydrophobic protective layer on the mucosa of the small intestine. Cholesterol was formed in the liver and used as the source of dietary fat which was absorbed by the small intestine of chylomicrons form. Fat absorption which occurs actively for triglycerides, cholesterol and phospholipids is formed in the gut, then the flow of blood to further join the protein (apoprotein) forming a lipoprotein that can circulate in the blood circulation [1].

D. *Triglycerides*

The average of trygliseride level was not affected by traetments. Triglyceride of TMR diet averaged 70.14 ± 6.76 mg/dl and triglyceride of RPC diet averaged 69.16 ± 3.53 mg/dl (showed in Table IV.). Triglyceride with RPC diet decreased 0.98 ± 3.23 md/dl (P = 0.77) than TMR diet although it was not affected significantly. Triglyceride level of two treatments were in the normal value that ranged 63 -77 mg/dl [20]. Choline forms phospatidylcholine of lipoprotein as phospholipids form so that can help the mobilization and β oksidation in liver which made trygliceride level in the blood lowest because more of lipoprotein produced. Addition choline chloride in feed increased NEFA mobilization to make taking up fatty acids by liver for oxidazing into ketone bodies and carbondioxide or trygliseride increased [4]. Lipoprotein contained of trygliceride, fatty acids, and fosfolipids so that lipoprotein was produced more, then made the more higher of trygliceride which was brought by lipoprotein [5].

E. *Low density lipoprotein*

The average of LDL level were not affected significantly (showed in Table IV.) and averaged 74.77 ± 12 mg/dl of TMR diet and 80.21 ± 16 mg/dl of RPC diet. LDL level with RPC diet was higher 5.44 ± 0.4 mg/dl (P = 0.35) than TMR diet including in the range of normal value eventhough it was not affected significantly. It indicated cause by increasing fat metabolism and role of choline as phosphatidylcholine that improve secretion of NEFA and triglycerides in VLDL then it will became to be LDL. LDL is a lipoprotein which has a lower density, serves a major carrier of essential fatty acids from the liver to the tissue for cells absorption and metabolism [19]. Normal levels of LDL in dairy cows ranged between 60 - 130 mg/dl and contained of triglycerides 10% and cholesterol

50% [21]. Addition 45 g/head/d of RPC (25% choline chloride) increased the de novo synthesis which was shown by the present of highly milk fatty acids [23]. Highly milk fatty acids due to the influence of choline caused by high level fatty acids in blood from being transported through VLDL. The addition of RPC 75 g/head/d (contained 37.5% choline chloride) increased the synthesis and secretion NEFA through VLDL from liver [14]. Methionine is supplied by choline that provids the amino acid profile of VLDL and phosphatidylcholine (main phospholipids) surface layer of VLDL that plays a role in the lipid transport [8].

F. High density lipoprotein

The average of HDL level in blood were not affected significantly by treatments (has been shown in Table IV.). HDL level of TMR diet averaged 70.52 ± 17 mg/dl and HDL level of RPC diet averaged 67.48 ±25 mg/dl. HDL level of two treatments was in the normal range of dairy cow's HDL level which was 40-90 mg/dl. HDL is a lipoprotein with low cholesterol levels and higher protein levels and it function plays in cholesterol transport from tissues to the liver. HDL is known as a molecule composed of fat (13% of cholesterol and triglycerides less than 5%) and 50% protein [21]. HDL level with RPC diet was lower 3.04 ± 0.8 mg/dl (P = 0.37) than TMR diet including in the normal value eventhough not affected significantly. The function of choline as phosphatidylcholine in bonding lipoprotein HDL mechanism has not been optimal because choline was more usable for chatabolism in order to produce energy than anabolism to form HDL. High of EEI caused fatty acid in blood higher so that made LDL level higher than HDL level. The nutrients from the food begins to be absorbed by the small intestine and then carried by the blood stream to the liver. HDL plays an important role in the mobilization of NEFA from the feed to be brought to the liver in lipoprotein formed, HDL. Choline is a precursor of phosphatidylcholine, a major phospholipid in high-density lipoprotein or HDL [24]. The function of lipoproteins is to transport cholesterol from peripheral tissues and from other lipoproteins to be oxidixed in the liver so that HDL plays a role in to prevent of atherosclerosis in blood vessels [22].

G. Milk yield

Milk yield was significant affected by treatments (has been shown in Table 3.) Milk yield of TMR diet averaged 13.84 ± 2.41 kg/d and the milk yield of RPC diet averaged 14.29 ± 2.90 kg/d (P = 0.05). The averages of milk yield in 4% FCM (Fat corrected milk) convertion of TMR diet was 13.36 ± 2.63 kg/day and RPC diet was 14.53 ± 3.12 kg/day (P = 0.04) (Showed in Table V.). It showed that increasing milk yield was made by choline that used for energy metabolism especially in chatabolism to produce energy for milk yield. Choline contributes in increasing energy eficiency and play as methyl group donor in energy metabolism, especially in lipid metabolism. Choline improved chatabolism of fatty acids and amino acids to produce Asetil CoA so it was obtained not only from glicolysis but also gluconeogenesis. Deposite of fatty acids increased while increasing choline chloride addition in certain level, it made gluconeogenesis better that improved glycogen from glycolysis to use for glucose source [3]. Asetyl CoA that produced by glycolysis and gluconeogenesis used to produced energy which used for milk yield. Glycogen increased while increasing of RPC consumption in dairy cow's liver. The slightly lower triglyceride concentrations for cows that received increasing amounts of RPC may have allowed for greater rates of gluconeogenesis, sparing glycogen from hydrolysis for use as a glucose source or perhaps replenishing glycogen in liver more quickly than cows not receiving RPC [14]. Addition RPC 12 g/head/d increased milk yield 3.6 kg [9], addition RPC 45 g/head/d which consists of 20 g choline chloride) in feed increased milk yield 2.9 kg [16] and addition RPC 30 g/head/d which consists of 37.5% choline chloride increased milk yield 0.3 kg than without addition choline chloride [23].

H. Milk fat

The average of milk fat was significant affected by RPC diet (showed in Table V.) Milk fat of TMR diet averaged 3.76 ± 0.27% and RPC diet averaged 4.11 ± 0.28%. RPC diet increased significantly 0,35% ± 0,01% of milk fat (P = 0.03). The averages of milk fat include in normal value of dairy cow's milk fat that ranged 3.24-4.49%. Milk fat increased because lipid metabolism in liver increased. LDL which was precursor of milk fat synthesis was higher with RPC diet than TMR diet. Mobilization of NEFA to take fatty acids by liver for oxidize into ketone and carbondioxide or tryglyseride increased [4]. Addition RPC increased cholesterol level 30% so lipid storage and lipid metabolism in balance by liver reached. Choline play role as methyl group donor in carnitine synthesis those use for lipid oxidation and lipotropic effect (Forms NEFA into tryglyserides and secretion tyglycerides) [17]. Milk fat increased because LDL which was as a precursor of milk fat tented to increase so lipid was carried into mammary gland quite a lot. Choline as phosphatidylcholine in lipoprotein bond made lipoprotein was more produced so that lipid esterification and tryglicerides from liver increased then carried as LDL into mammary gland. It implicated to increase mechanism in mammary gland such as milk yield and milk fat. Choline also play role as fosfolipid component in fat globule of milk fat so fluidity and rigidity from fat globule of milk depended by its [8].

IV. Conclusion

Choline Chloride as Rumen Protected Choline could be added in the diets of lactation cows to increase milk yield and milk fat.

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References

[1] S. Agustina, "Utilization of shrimp shell chitosan as reduce blood cholesterol levels in rat Sprague dawley," M.S. thesis, Dept. Applied Chem. Stud., Udayana Univ., Denpasar, Indonesia, 2015.

[2] A. Astuti. (June 2009). The effect of high quality feed supplement addition on the nutrient consumption and digestibility of early lactating dairy cow. *Buletin Peternakan.* [Online]. 33(2). pp. 81-87. Available:

http://journal.ugm.ac.id/index.php/buletinpeternakan/article/download/120/476

[3] H. M. Awwad, S. H. Kirsch, J. Geisel, and R. Obeid. (February 2014). Measurement of concentrations of whole blood levels of choline, betaine, and dimethylglycine and their relations to plasma levels. *Journal of Chromatogr B*. [online]. 975 (2014). pp. 41-45. Available: www.elsevier.com/locate/chromb https://doi.org/10.1016/j.jchromb.2014.02.030

[4] A. Baldi and L. Pinotti, "Choline metabolism in high-producing dairy cows: metabolic and nutritional basis," *in the Canadian Society of Animal Science Symposium. 2005* ,pp. 207-212, vol. 86, USA : Canadian Journal of Anim. Sci., 2006. https://doi.org/10.4141/a05-061

[5] W. W. Christie, *Lipid Metabolism in Ruminant Animals,* 1st ed. Headington Hill Hall, U.K.: Oxford, 1981, ch. 2, pp. 48-50.

[6] Y. H. Chung, N. E. Brown, C. M. Martinez, T. W. Cassidy, and G. A. Varga. (February 2009). Effects of rumen protected choline and dry propylene glycol on feed intake and blood parameters for Holstein dairy cows in early lactation. *J. Dairy Sci.* [Online]. 92(6). pp. 2729-2736. Avaiable: http://www.journalofdairyscience.org/article/S0022-0302(09)70590-9/fulltext?mobileUi=0

[7] S. Davidson, B. Hopkins. J. Odle. C. Brownie, V. Fellner, and L. Whitlow. (2008). Supplementing limited methionine diets with rumen-protected methionine, betaine, and choline in early lactation Holstein cows. *J. Dairy Sci* [Online]. 91(4). pp.1552–1559. Avaiable: http://agri.lu.ac.ir/uploads/PIIS0022030208712839_3751.pdf https://doi.org/10.3168/jds.2007-0721

[8] V. M. A. Etcheverry, "Absorbtion and utilization of choline and vitamin b12 in lactating dairy cows using different delivery methods," Ph.D. dissertation, Dept. Agric., Tennessee Univ., Knoxville, USA, 2014.

[9] J. R. Hartwell, M. J. Cecava, and S. S. Donkin. (October 2000). Impact of dietary rumen undegradable protein and rumen-protected choline on intake, peripartum liver triacylglyceride, plasma metabolites, and milk production in transition dairy cows. *J. Dairy Sci.* [Online]. 83(12). pp. 2907-2917. Avaiable: http://www.sciencedirect.com/science/article/pii/S0022030200751915

[10] D. B. Marks, A. D. Marks, and C. M. Smith. *Basic medical biochemistry: a clinical approach*, 1st ed. Lippincot Williams and Wilkins, US: Philadelphia, 1996.

[11] R. K. Murray, D. K. Granner, P. A. Mayes, and V. W. Rodwell, *Harper Biochemistr.* 25th ed. Stamford, CT: Appleton and Lange; 2003.

[12] National Research Council, *Nutrient Requirements of Dairy Cattle Seventh Revised Edition*, Rev. ed. National Academy Press, USA: Washington D.C.

[13] M. Parakkasi, *Ruminant Nutrition and Feed Science*, Indonesia University Press, Indonesia : Jakarta, 1999.

[14] M. Piepenbrink and T. Overton. (November 2003). Liver metabolism and production of cows fed increasing amounts of rumen-protected choline during the periparturient period. *J. Dairy Sci.* [Online]. 86(5). Pp. 1722-1733. Avaiable: https://www.ncbi.nlm.nih.gov/pubmed/12778583

[15] L. Pinotti, A. Baldi, and V. Dell'Orto. (October 2002). Comparative memmalian choline metabolism with emphasis on the high-yielding dairy cow. *Nut. Reserc. Rev.*[Online]. 047(15). pp.315-331. https://doi.org/10.1079/NRR200247

[16] L. Pinotti, A. Baldi, I. Politis, R. Rebucci, L. Sangalli, and V. Dell'Orto. (2003). Rumen-protected choline administration to transition cows: effects on milk production and vitamin e status. *J. Veterinary Med. series A.* [Online]. 50(1). pp.18-21. https://doi.org/10.1046/j.1439-0442.2003.00502.x

[17] L. Pinotti, A. Campagnoli, L. Sangalli, R. Rebucci, V. Dell'Orto, and A. Baldi. (2004). Metabolism of periparturient dairy cows fed rumen-protected choline. *J. Anim. Feed Sci.* [Online]. 13(1). pp.551–554.

[18] K. Sejrsen, T. Hvelplund, and M. O. Nielsen. *Ruminant Physiology*. Wageningen Academic Publishers, UK: Netherland, 2008.

[19] R. Soegih. (1995). Gangguan Penyakit Jantung dan Pembuluh Darah. Sadar Pangan dan Gizi. *Bulettin.* [Online]. 4(3). pp.2-3.

[20] M. M., Tighe and M. Brown. *Comprehensive Review for Veterinary Tehnicians*. Mosby Elsevier, USA: St. Louis, 2008.

[21] D. Weatherby and S. Ferguson. *Blood Chemistry and CBC Analysis-Clinical Laboratory Testing from a Functional Perspective*. Bear Mountain Publishing, USA: Ashland, 2002.

[22] Widiyanto, M. Soejono, H. Hartadi, dan Z. Bachrudin. (2007). Effect of protected kapok seed oil supplementation on in vitro ruminal lipid status. *Anim. Prod.* [Online]. 11(2). pp.122-128

[23] G. Xu, J. An Ye, L. Jianxin and Y. Yu. (March 2005). Effect of rumen-protected choline addition on milk performance and blood metabolic parameters in transition dairy cows. *J. Anim. Sci.* [Online]. 19(1). pp.390-395.

[24] S. H. Zeizel and K. A. da Costa. (2009). Choline: An Essensial Nutrient for Public Health. *Nutrition Review*. [Online] 67(11). pp.615-623. https://doi.org/10.1111/j.1753-4887.2009.00246.x

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